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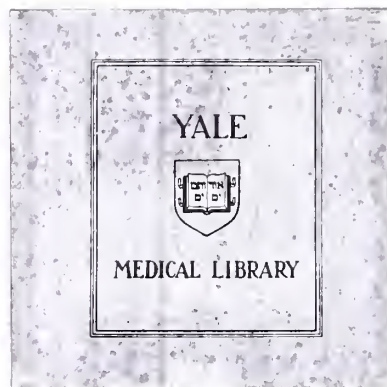
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CIRCULATING IMMUNE COMPLEXES IN LYME ARTHRITIS

THOMAS TRUMBLE

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CIRCULATING IMMUNE COMPLEXES IN LYME ARTHRITIS

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A THESIS SUBMITTED TO THE YALE UNIVERSITY SCHOOL OF MEDICINE
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF MEDICINE

1979

DEDICATION

-- to my parents.

ACKNOWLEDGEMENTS

This thesis could not have been possible without the advice and support of many individuals whom I would like to acknowledge. Throughout this research project the encouragement and insight of my advisor, John Hardin, and the technical expertise of Joan Carboni were invaluable. I would also like to express my appreciation to Elise DeSanna for her excellent preparation of this manuscript and to Stella Cretella for her help with this project.

INTRODUCTION

In this thesis I have demonstrated that circulating immune complexes occur in patients with Lyme arthritis. These complexes have a number of special features that are of particular interest to rheumatologists and clinical immunologists as they search for the cause of rheumatoid arthritis. Nearly all patients at the onset of disease had these immune complexes which became less prevalent with time and rarely present during periods of remissions. They contained Clq reactive IgG, had a density near 1.9S, and were partially cryoprecipitable. It is likely that they contained an antigen related to the causative agent of Lyme arthritis.

PRELUDE

The search for the causative agent of Lyme arthritis has involved the Yale University School of Medicine, the United States Public Health Service, and the Connecticut State Health Department. The initial investigation of the epidemiology and the clinical manifestations of Lyme arthritis was conducted by the Rheumatology Section of the Department of Medicine, the Department of Pediatrics, and the Department of Epidemiology and Public Health at Yale University School of Medicine. The methods for conducting this investigation were approved by the Human Investigation Committee (Protocol #1125). Further investigations were greatly aided by Dr. S. Ruddy from the Medical College of Virginia who measured serum complement levels and Drs. L. C. Walker and R. C. Williams from the University of New Mexico School of Medicine who tested serum samples using the Raji cell and Clq solid phase assays.

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I. LITERATURE REVIEW

A. LYME ARTHRITIS

Lyme arthritis is a new disease which was first recognized in 1975. Retrospectively no cases have been identified prior to 1972 (1). The initial clue that led to the discovery of this disease came from two women who, concerned about recent attacks of arthritis among their children, independently called the Connecticut State Health Department to report that they believed an epidemic of juvenile rheumatoid arthritis (JRA) was occurring in their small community of Lyme, Connecticut (1). Health Department officials subsequently contacted members of the Rheumatology Section at Yale University School of Medicine. This series of events triggered a retrospective study by members of the Yale Rheumatology Section to identify all residents of Lyme, Connecticut and neighboring communities with acute onset of arthritis.

In this initial retrospective study of 51 patients, Lyme arthritis was defined as a new clinical entity characterized by oligoarticular arthritis and geographical clustering. Although previously misdiagnosed as juvenile rheumatoid arthritis or rheumatoid arthritis (RA), Lyme arthritis was distinct because of the geographical clustering and the seasonal onset of the arthritis, June to September. About one quarter of the patients reported an expanding annular rash before the onset of arthritis. Nearly all of the 51 patients resided within one of three communities in eastern Connecticut: Lyme, Old Lyme, and East Haddam. One in ten children living on four country roads were affected.

Since Lyme arthritis occurred seasonally, Steere et al (2) began a prospective study of 32 patients with new onset of disease in 1976. In this study the skin lesion, erythema chronicum migrans (ECM), was clinically the most diagnostic feature of Lyme arthritis (2). Although this unique skin lesion has been observed in the U.S.A. on only a single occasion (3), multiple reports have described its occurrence in Europe where it has been associated with neurological symptoms but not with arthritis (4-10). Lyme arthritis patients have had neurological symptoms -- peripheral and central nervous systems -- in addition to cardiac symptomatology.

Skin: The initial lesion, ECM, was reported by about one third of patients to begin at the site of a tick bite. The lesion first appeared as a small red macule that expanded to form a large annular rash with an intense red border, partial central clearing, and an indurated center. After the initial lesion, some patients developed multiple smaller satellite lesions without an associated tick bite and without central induration. Scaling was not prominent and mucosal surfaces were not involved. Malaise and fatigue, chills and fever, headache and stiff neck were the most common symptoms associated with or preceding the skin lesion. On physical examination, patients often had elevated temperatures and less frequently lymphadenopathy, splenomegaly, malar rash, and periorbital edema.

Skin biopsy specimens from the center of the lesion showed heavy mononuclear cell infiltrates around blood vessels and skin appendages throughout the dermis. Thickened keratin, edema, and hemosiderin deposits were characteristic of epidermal changes. Unlike the center the outer border of the lesion showed only dermal changes.

Joints: Of the prospectively studied patients, 27 were studied from the beginning of their joint symptoms or before. These patients with arthritis frequently (19 of 27, 70%) had the onset of ECM a median of 4 weeks (0.6 - 22 weeks) previously. Patients usually noticed the sudden onset of an asymmetric arthritis in a few joints, most frequently the knee. However, some patients had migratory arthralgias. The duration of the arthritis was characteristically short (median 8 days), separated by longer periods of remission. At examination, affected knees were typically hot, but rarely red, and often very swollen with occasional formation and rupture of Baker's cysts. The synovium in Lyme arthritis had changes identical to those of rheumatoid arthritis, namely hypertrophy, vascular proliferation, and mononuclear cell infiltration.

Central Nervous System: As many as one third of the prospectively studied patients had symptoms suggestive of aseptic meningitis -- fever, headache, and stiff neck during the first few days of the illness. Two patients had severe recurrent headaches and stiff necks with lymphocytic meningitis confirmed by lumbar puncture.

Peripheral Nervous System: In addition to arthritis, three patients developed cranial nerve palsies. Of the two patients with Bell's palsy, one had a sensory radiculopathy over the T10 dermatome. The third patient had unilateral ptosis in addition to lymphocytic meningitis. The abnormalities commonly lasted for months but usually resolved completely.

Heart: After the skin lesion some patients developed myocardial conduction abnormalities such as first degree, Wenckebach, third degree atrioventricular, and alternating bundle branch blocks. Patients had reduced cardiac ejection fraction by Technetium sulfur colloid scans at the time of their conduction defects. None of the patients had evidence

of streptococcal infection although two did satisfy the Jones's criteria for rheumatic fever.

Etiology: Occasional reports have suggested that patients with ECM have antibodies against viruses (9, 11) or rickettsia (12, 13) but they have not been confirmed in subsequent studies (14, 15). Despite the use of extensive methods no viruses or bacteria were detected in cultures of biological samples from patients with Lyme arthritis, and serologic tests -- including tests for 216 arboviruses, 38 of which are known to be carried by ticks -- did not suggest infection with agents known to cause other forms of arthritis. Serum analysis for rheumatoid factor (RF) and antinuclear antibodies (ANA) were almost invariably negative (2).

Epidemiology: The three affected Connecticut communities were sparsely populated areas bordering the Connecticut River. Most patients in these communities lived on large wooded lots or farms. A tick bite was recalled at the site of the lesion by several patients, and epidemiological analysis of the arthritis clusters suggested transmission of a causative agent by an arthropod vector (1, 2, 16). The location of the lesion on the trunk and proximal extremities, also favored a crawling arthropod. Unlike the pattern of airborne spread, family members usually had onset of the disease during different years (1, 2). The time of onset of ECM, from May to August, was also consistent with an arthropod vector (2).

Lyme arthritis has resembled immune complex disorders in clinical manifestations of disease and in clinical response to steroid treatment. Furthermore, sera from patients with ECM and cryoglobulins had lower complement levels and were more likely to develop arthritis than patients

with ECM alone (2, 17). These findings were indirect evidence that immune complexes might play a role in the pathogenesis of Lyme arthritis.

B. IMMUNE COMPLEXES AS MEDIATORS OF TISSUE INJURY

Immune complexes are defined as the combination of an antibody and an antigen. The nature and the quantity of immune complexes are dependent on the dynamics of formation, clearance mechanisms, and tissue deposition. The formation of these complexes is part of normal physiology but the untoward activation of immune mechanisms may lead to disease.

As summarized by Caldwell (18), the formation of immune complexes depends on: number of binding sites available, antibody affinity, and antigen to antibody ratio. The number of binding sites depends on the valency of the antibody and the number of determinants on the antigen. The normal antibody affinity varies 10^{12} to 10^4 liters per mole. Antibody affinity depends on the type of immunoglobulins (i.e. IgG has greater affinity than IgM) and the temperature. (Cold-antibodies have increased affinity for antigen at lower temperatures.) Complexes formed near the equivalence point, 1:1 ratio of antigen and antibody, have greater lattice formation and therefore greater size than complexes formed at other ratios. The complexes may be modified after formation by complement and by cells which bind to the Fc receptor of immunoglobulins.

Immune complexes are formed in circulating blood or in tissues. The large circulating complexes fix complement and are readily cleared by the phagocyte system (19). Small complexes are less efficiently cleared -- to some extent by the spleen -- or they are deposited in renal glomeruli, blood vessel walls, or choroid plexus (20, 21).

Immune complexes can form in tissue by several mechanisms. In the Arthus type reaction, antigen present locally reacts with circulating antibody. The immune complexes involved in the glomerulonephritis of Goodpasture's syndrome are formed by circulating antibody reacting with

glomerular basement membrane (GBM) as the tissue bound antigen (22).

Antibody and antigen can both be released locally to form immune complexes, i.e. late granulomas with shistome eggs (23).

Immune complexes with the greatest pathogenetic potential are primarily those that can activate complement or react with various cell types via immunoglobulin (Fc) or complement receptors. Following immune complex binding to Fc receptors, cell mediated tissue damage results from phagocytosis (24) or exocytosis with release of vasoactive amines (25). Immune complex binding of complement triggers the production of activated complement components which can lyse cells (26, 27) or recruit leukocytes to cause tissue injury (22, 25, 28).

The importance of complement activation as a cause of tissue damage can be demonstrated in vivo, for instance, inflammation of an Arthus reaction can be prevented by prior C3 depletion with cobra venom. Cell lysis results when immune complexes are brought in close apposition with cell membranes or are formed between antibody and a cell membrane bound antigen. The terminal components can directly lyse the cell or indirectly lyse a bystander cell (26, 27). Activated complement components can recruit leukocytes by initiating leukocyte migration (components C5⁶⁷ and C5a [22], binding (component C3b [28], and exocytosis (components C3b and C5a [25]).

Tissue damage also results from immune complex activation of leukocytes, platelets, and lymphocytes via the Fc receptor. Neutrophilic phagocytosis is initiated by only those complexes formed between an antigen and IgG1, and IgG3. All subgroups of IgG in addition to IgA can stimulate neutrophilic exocytosis with release of granules containing proteolytic enzymes

and basic peptides (25). These enzymes and peptides result in increased vascular permeability and activation of the clotting system (25). Johnson et al (29) used millipore filters coated with IgG as a model of immune complexes to show that the release of toxic oxygen metabolites and release of granules are stimulated by immune complexes. The factors released by neutrophil exocytosis probably result in much of the tissue injury that occurs in human inflammation; dependence upon neutrophils for the development of the inflammatory manifestations of serum sickness arteritis (28, 29), Arthus reactions (30, 31) and antibody mediated nephritis (32) has been described.

The interaction of immune complexes with macrophages has only recently been studied although they are a constant feature of inflammatory reactions (33). Mononuclear phagocytes have receptors which have greater affinity for the Fc region of IgG1 and IgG3 than IgG2 or IgG4; attachment of antibody-coated cells to these receptors is followed by phagocytosis (24). Similar to neutrophils, the exposure of macrophages to immune complexes is accompanied by selective release of macrophage lysosomes (34). Moreover, the ability of macrophages to contribute to inflammation, i.e. in arthritis (35), has been demonstrated in animal models.

Basophiles and mast cells, however, require immune complexes with IgE and to a lesser extent IgG to stimulate exocytosis (36). Basophile granules contain vasoactive substances (slow reacting substance of anaphylaxis, and eosinophilic chemotactic factor of anaphylaxis), factors affecting the clotting system, and platelet activating factor (36). An IgE-mediated anaphylactic response often accompanies the deposition of immune complexes and appears to be responsible for the deposition in arteries and glomeruli (20).

Human platelets bear Fc receptors for IgG 1, 2, 3 and 4 but not for other classes of immunoglobulin or for complement (25). Immune complexes bind via this receptor to induce platelet clumping and release of vasoactive amines (25). These amines increase vascular permeability which may be essential in allowing circulating immune complexes to become trapped in vessel walls (20).

Human peripheral blood lymphocyte cells are a heterogenous population consisting of T cells which mediate cellular immunity, B cells which mediate humoral immunity, and a third cell population defined by its ability to lyse target cells coated with antibodies (37, 38). This third population, generally referred to as killer or K cells, may cause tissue destruction by the mechanism of antibody-dependent cell-mediated cytotoxicity. K cells appear morphologically similar to small lymphocytes but they lack the surface markers normally associated with mature T and B cells (36). Instead, K cells possess receptors for the Fc portion of IgG and for C3 (37). In order to lyse target cells, K cells must bind to the Fc receptor of antibodies attached to the target cell. This mechanism of tissue destruction has been demonstrated to occur in autoimmune diseases such as Hashimoto's thyroiditis (38, 39).

C. IMMUNE COMPLEXES IN HUMAN DISEASE

The clinical features of immune complex disease depend on the position and the deposition of the complexes. The composition of the complexes varies with the nature of the antibodies and antigens involved. In systemic lupus erythematosus for instance, evidence from animal models suggests that antibodies with weak affinity are formed (40); the low affinity antibody results in the formation of complexes which are more likely to escape the phagocyte system (41). The affinity of DNA for collagen (42) may explain the glomerular membrane deposits of DNA-antiDNA and therefore the nephritis in systemic lupus erythematosus. The deposition of immune complexes depends on whether they are formed in body fluids as circulating immune complexes or in body tissues. Circulating immune complexes are either cleared by the mononuclear phagocyte system or deposited in renal glomeruli, choroid plexus or vessel walls (20, 21). Circulating complexes which are small (19), which do not bind complement (19) or which are composed of antibodies with low affinity for antigen (41) are more likely to evade the phagocyte system and be deposited. Immune complexes which are formed in tissues result in an Arthus reaction, and the site of inflammation will vary with the method of antigen deposition, i.e. injected into the skin, inhaled into the lungs, released into tissues by parasites, etc. (43).

Diseases associated with immune complexes are a very large and diversified group. They are most conveniently categorized by the origin of the antigen, endogenous or exogenous. Endogenous antigens include IgG of rheumatoid arthritis, nuclear antigens of systemic lupus erythematosus, and cell specific antigens of tumors and autoimmune diseases. Among the

exogenous antigens are infectious agents (viruses, bacteria, etc.), environmental causes (pollens, dusts, etc.) and introgenic causes (the proteins of serum sickness, drugs, etc.). Well studied and representative immune complex diseases that are pertinent to this thesis include rheumatoid arthritis, systemic lupus erythematosus, hepatitis B, and dengue hemorrhagic fever.

Rheumatoid Arthritis: Clinically, rheumatoid arthritis is characterized by destructive joint disease with less frequent systemic manifestations, i.e. subcutaneous nodules, vasculitis, pulmonary fibrosis, and pericarditis but rarely kidney lesions. The arthritis usually involves the small joints in a symmetrical fashion. The incidence of rheumatoid arthritis increases with age, and is sex related because women are affected twice as frequently as men.

The formation of immune complexes and the activation of the complement system are known features of rheumatoid arthritis. Many of the techniques -- both direct and indirect -- for the study of immune complexes were developed in the search for the pathological mechanism of the disease. Immunofluorescent staining has provided indirect evidence of immune complexes in tissues. Using these staining techniques, Fish (44) and Rodman (45) demonstrated IgG, IgM and complement in synovial membranes of patients with rheumatoid arthritis. Similar deposits have been found in rheumatoid nodules (46), nerve tissue (47) and vessel walls (48). Subsequently, Franco and Schur (49) and Hunder and McDuffie (41) reported low complement levels in rheumatoid arthritis patients, evidence which suggested complement consumption by immune aggregates.

Direct evidence of the presence of immune complexes in rheumatoid arthritis had been provided as early as 1957 by Franklin et al (51) who

demonstrated by analytical ultracentrifugation the presence of a high molecular weight material (22S) in the serum of rheumatoid arthritis patients. That immune complexes possessing biological activity might be present in rheumatoid arthritis has been shown by Baumal and Broder (52) who isolated high molecular weight material, rheumatoid biologically active factor (RBAF), capable of initiating a complement dependent reaction resulting in the release of histamine from guinea pig lung. This material, RBAF, was present in sera and joint fluid, and it correlated with disease activity (53). A simpler technique for demonstration of immune complexes was introduced in 1967 by Hamstead (54). He found that certain high-titer rheumatoid factors (RFs) capable of precipitating aggregated IgG, produced precipitin reactions with rheumatoid synovial fluid. Winchester et al (55) reported that monoclonal rheumatoid factors (mRFs) were superior for demonstrating this material.

All these techniques have certain drawbacks that limit their usefulness in studying the relationship of immune complex-like material to disease. Analytical ultracentrifugation is relatively insensitive and too cumbersome for analysis of larger numbers of samples. The bioassay technique of Baumal and Broder is very complex and undoubtedly will prove difficult to standardize. Immunodiffusion is nonquantitative and weak bands are difficult to determine with confidence. Therefore, investigators have developed a new generation of assays involving radioimmunoassays, i. e. the ^{125}I -Clq binding assay by Zubler et al (46) and the mRF assay by Luthra et al (47).

Rheumatoid arthritis patients more frequently had positive tests with the ^{125}I -Clq binding assay, 76% of synovial fluids and 63% of sera,

than with the other assays (56, 57, 58). The Clq reactive material sedimented with a density of 10-34S with the maximum amount of material sedimenting at 14-15S (56). There was no correlation between the amount of immune complexes detected in the assays and RF titers (56, 57, 58). However, the results of the assays did show a negative correlation with the levels of complement component C4 (56, 57).

Although C4 levels were often depressed to the lower limits of normal in rheumatoid arthritis patients, the levels of most complement components have been generally normal or elevated in this disease (59, 60, 61). Elevated complement levels might be explained by an increased synthesis of complement which is often seen in inflammatory disease (61) probably associated with increased catabolism as suggested by radio-labelled turnover studies of complement (62). Nydegger et al (63) found elevated levels of C3d in the sera and joint fluids of patients with rheumatoid arthritis which correlated with the amount of Clq reactive material in patient materials but not with RF titers. These results strongly suggest the presence of immune complexes and the activation of complement in these biological fluids. The C3d levels in synovial fluid were higher than and only poorly correlated with the C3d level in serum. This observation was consistent with parallel activation of complement in synovial fluid and serum.

The major antigen in immune complexes of rheumatoid arthritis was assumed to be IgG but this theory is now questionable. Synovial lymphocytes produce an IgG that is recognized as foreign by the body (64). Rheumatoid factors (RFs), IgM type anti-IgG and IgG type anti-IgG, are produced in response to the antigenically active IgG, IgG*, immune complexes then form between RF and IgG* (64). Studies of IgG* have

demonstrated that a defect exists in the hinge region of the immunoglobulin and that the defect is also responsible for the abnormal avidity of IgG*for B lymphocytes (65). In some lymphoproliferative diseases a monoclonal rheumatoid factor (mRF) of the IgM type is produced. The IgM RF can bind up to five IgG*s to form complexes thought to have pathological potential. However, the participation of 19S RF in at least complexes that bind Clq appears limited since the concentration of the Clq reactive material does not correlate with RF titers and the density of the Clq reactive material, 14-15S, is less than that of IgM RF (56). Some investigators have suggested that RF has a role in the pathogenesis of rheumatoid arthritis by enhancing the antibody-dependent cell-mediated cytotoxicity (ADCC) (65). However, Feldmann et al (66) have found the ADCC to be normal in rheumatoid arthritis.

Other materials such as cell proteins, collagen, and virus particles have been implicated as the antigen in the immune complexes of rheumatoid arthritis. Some evidence indicates that proteins in lymphocyte membranes or nucleoproteins may have antigen activity in rheumatoid arthritis (67). Many investigators have suggested that collagen is an important antigen in rheumatoid arthritis. Evidence by Steffen et al (68) demonstrates that anticollagen antibodies are present in more than one third of sera from patients with rheumatoid arthritis. Using collagen digesting techniques, Mentzel et al (69) has shown that collagen-anticollagen antibodies are present in the synovial fluids of rheumatoid arthritis patients. An animal model of arthritis can be produced by the injection of collagen into the knee joints of collagen immunized animals (70). Viruses have also been implicated in the disease because of the decreased sensitivity of rheumatoid synovial cells to infection by Newcastle and rubeolla

viruses (67).

Both the direct and indirect methods for detecting immune complexes have shown a correlation with disease activity. Though Zubler et al (56) did not find a correlation between the amount of Clq reactive material in serum and disease stage, he did find a positive correlation between the presence of systemic disease and the presence of Clq reactive material. Nydegger et al (63) found that the C3d levels correlated with a disease index which took into account the joint score, morning stiffness, and erythrocyte sedimentation rate. The difficulty in correlating disease activity with the concentration of reactive materials in the assays may result from the parallel activation of synovial fluid and serum. Furthermore, the production of immune complexes and the activation of complement in each joint is most likely independent from other joints. Therefore, assay results from synovial fluid obtained from one joint would not reflex the disease activity in other joints.

Systemic Lupus Erythematosus (SLE): Systemic lupus erythematosus is characterized by malar skin rash, arthritis, vasculitis, pleuritis, pericarditis and nephritis. The most frequent cause of death in this disease is the complications of renal failure. The clinical diagnosis of SLE is aided by positive LE preparations and antinuclear antibody (ANA) titers.

Indirect evidence of DNA-antiDNA complexes in renal glomeruli was demonstrated in 1967 by Koffler (71) using elution and immunohistochemical techniques. Atkins et al (72) demonstrated similar complexes in the choroid plexus of SLE patients. Additional evidence of immune complexes was demonstrated by Winchester et al (73) who showed a correlation between complement depletion and immune complexes in the sera and synovial fluids

of SLE patients.

Direct evidence of immune complexes in SLE was first provided by Agnello (74) who demonstrated Clq precipitation of immune complex-like material using immunodiffusion techniques. Recently developed radio-immunoassays, i.e. ^{125}I -Clq binding assay (75, 76), Clq deviation assay (58), mRF assay (58), and Raji cell assay (77) have frequently detected immune complexes in the sera of patients with SLE. All patients with SLE tested by the Raji cell assay had positive results (77) a larger percentage than the 49% reported by Gabriel using the Clq deviation assay (57). Results of both assays had positive correlations with ANA titers and negative correlations with complement levels (57, 77). Agnello et al (58) and Levinsky et al (78) found large and small complexes of 19S and 7S densities respectively. In addition, Levinsky et al (78) found intermediate size complexes which correlated with the presence of renal disease.

Evidence of complement activation has been demonstrated by Nydegger et al (75), Theofilopoulos et al (77), and Agnello et al (58). These investigators noted a negative correlation between the CH50 levels and the presence of immune complexes. Levinsky et al (78) also noted depressed levels of Clq in SLE patients. Perin et al (79) demonstrated elevated levels of C3d and Ba fragment of properdin indicating that classical and alternate pathways were involved probably resulting from immune complex activation.

Nuclear antigens have been suspected of being involved in immune complex formation since the discovery of LE preparations and their correlation with the diagnosis of SLE. The titers of ANA also have correlated with disease activity. Disease activity and positive tests

for immune complexes correlated best with the titers of anti-sDNA (75). The presence of antibodies to sDNA and DNA:RNA have suggested a viral etiology, and oncornavirus (80) and reovirus (81) have been implicated in this disease. However Cano et al (82) demonstrated that only 3 of 11 sera pretreated with DNase had lower amounts of Clq reactive material when compared to controls. This observation suggested that antigens other than DNA or RNA may be involved in systemic lupus erythematosus. Evidence to support this possibility has been the presence of several other auto-antibodies, i.e. antilymphocyte and anti-immunoglobulin antibodies (64).

Search for the cause for lymphocyte production of auto-antibodies has lead to the investigation of possible T and B cell defects in SLE. Many of these experiments have used New Zealand black and white mice (NZB and NZW respectively) because like SLE patients they have positive ANA titers and develop nephritis. Fye et al (64) have summarized the evidence implicating defective T cell function in systemic lupus erythematosus. These antibodies kill T cells or bind their receptors and block their function. This evidence correlated with the deficiency of suppressor T cells noted in NZB mice. Furthermore, T cell function decreases with age in these animals. New Zealand black mice treated with thymic hormone have prolonged T cell function and delayed onset of the nephritis. The decrease in suppressor T cells correlated with enhanced B cell activities in NZB mice. Mice treated with a lymphocytotoxic agent, L-asparaginase, have decreased numbers of B cells and delayed onset of the nephritis (83).

An abnormality of K cell function, the antibody-dependent cell-mediated cytotoxicity (ADCC), in patients with systemic lupus erythematosus has also been observed. There appeared to be a "blocking factor"

in patient sera which caused the decrease in the ADCC (66). This "blocking factor" was present in the same portion of sucrose gradients as immune complexes, and was removed when the serum was incubated with DNase and pronase or when IgG was removed -- features consistent with immune complexes (DNA-antiDNA) as the "blocking factor" (66). Moreover, the ADCC of patient sera correlated with the extent of disease activity (66). Whether the immune complex-mediated inhibition of ADCC is a specific factor in the pathogenesis of systemic lupus erythematosus or not has not been determined.

The disease activity of patients with systemic lupus erythematosus has correlated well with the evidence for immune complexes. The material reactive in the radioimmunoassays for immune complexes and the anti-DNA antibody titers both correlated with disease activity. Gabriel et al (58) studied systemic lupus erythematosus patients longitudinally and found that immune complex levels and anti-DNA titers rose and fell in parallel with the exacerbations and remissions of disease. Levinsky et al (78) found that the size of immune complexes correlated with the manifestation of disease; intermediate size complexes correlated with the presence of nephritis.

In addition to the presence of immune complexes, studies with animal models have indicated that the onset of disease also correlates with the decrease in the clearance of immune complexes by the mononuclear phagocyte system. Preceding the onset of nephritis in NZB mice, Petty and Steward (40) have demonstrated a decrease in the affinity of antibodies using the globin precipitation technique. Immune complexes composed of antibodies with low affinity for antigens would be less likely to be

cleared by the phagocyte system (41). Morgan and Steward (42) found that the macrophage clearance function of these mice also decreased prior to the onset of nephritis. The antibody affinity and the macrophage clearance might be key factors which determine whether or not circulating immune complexes will have pathogenic potential.

Hepatitis B: Patients with hepatitis B infection have been noted to have one of several clinical manifestations. Patients with mild disease have experienced transient urticarial skin rash, polyarthralgias and clinical jaundice. These patients frequently had cryoglobulins in their sera (84). The persistence of disease could result in symptoms similar to chronic serum sickness with renal (85) and vascular (86, 87) lesions in addition to liver injury.

In addition to direct evidence of immune complexes in serum hepatitis, laboratory evidence of immune complexes in this disease has also been provided by immunoelectronmicroscopy, complement studies, cryoglobulin studies and studies of antibody-dependent cell-mediated cytotoxicity (ADCC). Using electron microscopy, Almeida and Watterson (88) demonstrated the presence of immune complexes in the sera of individuals with chronic active or acute hepatitis. A pattern of immunofluorescence which indicated the presence of IgG, HBsAg and complement -- components likely to be involved in the formation of immune complexes -- has been identified on the hepatocyte membrane and in the cytoplasm of liver cells in patients with chronic active hepatitis (89). Gerber et al (90) used fluorescent antibody techniques to demonstrate that complexes of IgG and HBcAg were in the nuclei of hepatocytes from patients with chronic active hepatitis but not from patients with acute hepatitis. Deposits of HBsAg,

IgG and complement have been identified in vascular (91) and renal lesions (85) suggesting immune complex involvement in extrahepatic disease.

Serum from patients with hepatitis B infection and with decreased complement levels, suggestive of depressed complement levels, was reported by Thiry et al (92). While Fordor et al (93) did not find a significant loss of total complement, complement components C1 and C3 each exhibited titers below normal in about half of the patients with chronic active hepatitis.

Another method for identifying immune complexes in hepatitis B has been the study of cryoglobulins. Mixed IgG-IgM cryoglobulins in serum have been considered evidence of circulating immune complexes (94, 95). Fordor et al (93) and Carella et al (96) have reported that cryoglobulins are present in patients with serum hepatitis. The hepatitis B virus has been identified in the cryoprecipitates of serum from patients with this disease (97).

A recent correlation with the presence of immune complexes in hepatitis B infections has been the ADCC. Fye et al (98) demonstrated that the ADCC was decreased in patients with serum hepatitis and that the decrease in the ADCC correlated with the presence of immune complexes as detected by the Raji cell assay. The return to normal of the ADCC with overnight incubation of the lymphocytes (allowing regeneration of the surface membranes and shedding of the membrane-bound material) suggested that the defect was due to cell membrane bound material. Furthermore, this ADCC "blocking factor" was present in the same portion of density gradients as the immune complexes. It was hypothesized that the "blocking factor" was immune complexes that bound the Fc receptors on killer (K)

cells to prevent the binding to and lysis of the target cell. The role of these blocking factors in the pathogenesis of serum hepatitis, i.e. a disruption of the immunoregulatory system or just an epiphenomenon, has not yet been defined.

Direct evidence of immune complexes in the sera of patients with hepatitis B infection was provided by Collier et al (99) using a radio-immunoprecipitation assay. Using the Raji cell assay Theofilopoulos et al (77) reported the presence of immune complexes in 53% of sera from patients with serum hepatitis; similarly Nydegger et al (59) reported positive tests with the ^{125}I -C1q binding assay in 33% of patient sera. Theofilopoulos et al (77) demonstrated that fractionated sera from patients, with positive tests for Australian antigen and with negative tests for immune complexes, had HBsAg, IgG and C3 in the middle portion of the gradient. However, sera from patients, with positive tests for the Australian antigen and with positive tests for immune complexes, had HBsAg, IgG and complement in the middle and lower fractions. This data was consistent with immune complexes containing the Australian antigen in the sera of patients with hepatitis B.

Immune complexes important in the pathogenesis of serum hepatitis may form between one or more of the three antigens; HBsAg -- the virus coat protein, HGcAg -- the nucleocapside, and HBe; and their corresponding antibodies (100). Several studies have provided evidence to implicate HBsAg in the formation of immune complexes. Theofilopoulos et al (77) demonstrated the presence of HBsAg in the same portions of gradients of fractionated sera as immune complexes. In addition, immunofluorescent studies demonstrated HBsAg bound to Raji cells (77). Electronmicroscopic

studies have shown hepatocytes coated with HBsAg (89).

There is evidence that other antigens besides hepatitis B virus particles might be involved in the formation of immune complexes in serum hepatitis. Fodor et al (93) demonstrated that rheumatoid factors were present in the sera of about one third of patients with chronic active hepatitis. The presence of rheumatoid factor has suggested that an abnormal antigenically active IgG may be present. Moreover, other auto-antibodies, i.e. antinuclear, antimitochondrial and anti-smooth muscle antibodies, has indicated that additional antigens may be involved, but none of these humoral factors have liver specificity (93).

The stage of disease in patients with serum positive for the Australian antigen correlated with the reactivity of patient sera in assays for immune complexes. Theofilopoulos et al (77) demonstrated that serum from patients with acute disease had more reactive material in the Raji cell assay than serum from patients in the convalescent phase of the disease, and the sera from asymptomatic carriers rarely contained reactive material. The reactive material in the Raji cell assay showed a positive correlation with enzyme and bilirubin levels (77). The amount of free HBsAg has correlated inversely with the severity of hepatitis (101). The excess free antigen may have prevented immune complex formation between antibody and antigen on hepatocytes thereby avoiding hepatocellular damage (101).

Dengue Hemorrhagic Fever (DHF): Dengue hemorrhagic fever is a serious manifestation of infection by one of four types of Dengue virus -- a group B arbovirus. Shock, petechiae and proteinuria are the major features of this disease. The shock is the result of massive losses of

fluid, electrolytes, and colloid (102) and treatment with blood transfusions has reduced the fatality from 10% to 2%.

Immunopathological reactions suggested to play a role in the pathogenesis of DHF have been summarized by the World Health Organization (101). A clinical argument in support of this role is that the majority of affected children display evidence of prior dengue virus infection. Infants less than one year old have had evidence of primary dengue infection, and in these patients the presence of maternal antibody might be important. A mechanism has been suggested by which dengue virus replication might be amplified during a second infection. Antibody against one strain of virus does not neutralize a different strain of virus, but increases its capacity to replicate with monocytes because virus-antibody complexes would attach more readily to the monocyte through the Fc receptor than would the free virus.

Indirect evidence from immunohistochemical techniques and complement depletion studies and direct evidence from radioimmunoassays have implicated immune complexes in the pathogenesis of DHF. Characteristic of DHF is the massive activation of both classical and alternate complement pathways (103). Boonpucknavig et al (104) has demonstrated the presence of virus antigen, IgM, and C3 in the glomeruli of children with DHF and proteinuria. Patients with DHF frequently have maculopapular and petechial skin lesions which have been demonstrated to contain virus antigen, IgM, and C3 (101). Theofilopoulos et al (77) using the Raji cell and Sobel et al (105) using the Clq deviation assay have provided direct evidence of immune complexes in the sera of patients with DHF. Theofilopoulos et al (77) reported that 63% of sera obtained from patients

in various stages of DHF had positive results in the Raji cell assay.

The presence of immune complexes in DHF correlated with the stage of disease and the site of the lesion. Sobel et al (105) noted that the amount of Clq reactive material increased with disease activity. Theofilopoulos et al (77) demonstrated that sera from patients with more advanced stages of disease more frequently had evidence of immune complexes when compared with sera from patients in the earlier stages of disease. Moreover, patients had evidence of immune complexes at the site of both skin and renal lesions. The process by which the shock syndrome is produced has not been fully explained, but the action of immune complexes to cause the release of vasoactive amines from platelets might be an important factor.

In the summary of these immune complex disorders, the evidence suggests that investigative techniques detected immune complexes in patient materials and that the immune complexes were involved in the pathogenesis of the disease and not just an epiphenomenon. Proof that immune complexes were present in patients with disease has been provided by analysis of tissue and biological fluids from patients. Indirect evidence was demonstrated by immunohistochemical techniques showing the presence of immunoglobulins, complement and occasionally antigens in tissues. Complement studies showing depressed levels or elevated levels of breakdown products have indicated consumption by immune complexes. Results of assays for immune complexes have frequently been positive with patients with disease but were rarely positive in controls. The densities of material reactive in these assays, i.e. the 14-15S complexes of rheumatoid arthritis (77), have correlated with that of immunoglobulins in vitro.

Furthermore, antigens such as HBs in hepatitis B, have been detected in the same sucrose density fractions which have material reactive in the immune complex assays (77).

Studies of immune complex disorders have demonstrated positive correlations between the presence of immune complexes and the disease activity and between the size and the site of deposition of the complexes. Both clinical, i.e. the disease index of systemic lupus erythematosus, and the laboratory assessment of disease, i.e. the bilirubin and enzymes of hepatitis B, of disease activity have correlated with the amount of reactive material in the immune complex assays. Moreover, Feldmann et al (66) and Fye et al (98) have demonstrated that the presence of immune complexes and the progression of disease activity correlated with decreases in the ADCC -- suspected to be the result of a "blocking effect" of immune complexes. Whether this "blocking effect" alters the mechanism of immune complex-mediated tissue injury or not remains to be determined. Levinsky et al (78) demonstrated a correlation between the presence of complexes of intermediate size and the presence of nephritis in patients with systemic lupus erythematosus. Finally, deposits of immune complexes were frequently present at the site of inflammation, i.e. the glomeruli, joints, skin, and choroid plexus.

D. DETECTION OF IMMUNE COMPLEXES

Immune complexes may be present in serum, in synovial fluids, in other biological fluids, or in tissues; and methods suitable for the detection of the complexes at these various sites are often different. The tissue-bound complexes can be studied in tissues or removed and studied similar to circulating immune complexes using techniques that are either antigen-specific or antigen-nonspecific. When the antigen is unknown investigators must rely on assays which involve physical and biologic properties of immune complexes independent of the identity of the antigen.

Detection of Immune Complexes in Tissue

Immune complexes in tissues can be detected by techniques to visualize the complexes in the tissue or by methods to remove and quantitate either the antigen or the antibody. In hepatitis B, for instance, electronmicroscopy provides visualization of deposits of immune-complex like material consisting of HBs antigen, antibodies, and complement components (88). Indirect evidence of immune complexes in serum hepatitis and in other immune complex disorders can be obtained by techniques which stain tissues for immunoglobulin, antigen, or complement (89). Antigens can be removed from tissues using elution techniques with subsequent quantification of the free antigen (71). On the other hand, antibody can be released from tissue using antigen destroying techniques, i.e. DNase to release anti-DNA antibodies (106), and subsequently the freed antibody can be quantitated in order to estimate amount of complexes that were deposited in the tissue.

Detection of Immune Complexes in Biological Fluids

Identification and quantification of immune complexes in biological fluids are greatly aided when the specific antigen is known. For instance, radiolabelled antibody against the antigen will detect immune complexes once free antigen has been excluded. Successful separation of free and bound antigens has involved countercurrent immunoelectrophoresis (106). Free and complex-bound antigen can also be differentiated by measuring the amount of antigen that is removed from a biological sample when hosts' immunoglobulins are specifically precipitated or absorbed. In the case of lactic dehydrogenase virus, the infectivity of sera after precipitation of immunoglobulins (including immunoglobulin-bound viruses) decreases by an amount proportional to the amount of immune complexes (107).

Assays Involving Physical Properties

In most cases of immune complex disease, the antigen is not known and nonspecific techniques which use physical properties or biological activities of immune complexes are necessary. Immune complexes may be isolated by size using techniques such as ultracentrifugation with sucrose density gradients (108), gel chromatography (109), and ultrafiltration. The solubility of immune complexes is another property that distinguishes them from unbound immunoglobulins. Immune complexes often undergo cryoprecipitation, spontaneous precipitation at 0-4°C, and they can be differentiated from cryofibrins which are insoluble in acetate buffer unlike cryoglobulins. Polyethylene glycol (PEG), an uncharged linear polymer, will decrease solubility and thus will precipitate proteins depending on the molecular weight of the protein and the con-

centration of PEG (110). A 4% solution of PEG (MW = 6000) will precipitate immune complexes but not free immunoglobulins (75). These methods are good for analytical study of immune complex disease especially in detecting low affinity antigen-antibody reactions which are otherwise difficult to study (108), but they are of limited value in routine work.

Assays Involving Biological Properties

Biological assays make use of immune complex binding to complement receptors, Fc receptors or to other substances specific for immune complexes, i.e. bovine conglutinin and rheumatoid factor (RF). These assays are fast and well suited for studying immune complexes in many patient samples. The major drawbacks to these assays are the difficulty in standardizing the assays, especially assays involving the function of living cells, and the interference from other biologically active factors.

Assays Involving Complement Receptors

Assays involving complement receptors include direct methods to detect immune complexes using exogenous C1q binding to receptors in the immune complexes or involving complex-bound complement binding to receptors on cells and indirect techniques to demonstrate complement consumption by immune aggregates. Exogenous C1q can be used in direct binding or inhibition assays. Both types of C1q assays have broad specificity because any serum with immune complexes that bind C1q will have a positive test. However, this advantage is offset to a certain extent by the nonspecific reactivity of C1q with such substances as C-reactive protein, DNA, and endotoxin (111, 112, 113).

- The C1q agarose precipitation test is a direct binding, but nonquantitative, test which involves the precipitation of immune complexes with

Clq in agarose gel (74). Sera from patients with systemic lupus erythematosus reacts more frequently in this assay than does other pathological sera. However, polyanions such as DNA (which is frequently present in sera from patients with systemic lupus erythematosus) will bind Clq to interfere with this assay and produce false positives (74).

- The ^{125}I -Clq binding assay uses PEG to preferentially precipitate immune complexes after incubation with radiolabelled Clq (75). The addition of EDTA prior to the addition of ^{125}I -Clq obviates the need to heat inactivate complement in test sera (75). Furthermore, Clq will rarely precipitate with C-reactive protein, endotoxin, or DNA in the presence of EDTA (76, 114).

- The Clq solid phase uses Clq bound to polystyrene tubes prior to the addition of test sera. Radiolabeled staph protein A then binds to complexes which have bound to Clq (115). The staph protein A provides greater specificity than the previously used radiolabeled antihuman IgG provided (115). However, some specificity is sacrificed because staph protein A will predominantly bind complexes containing IgG1, or IgG2 (116).

- There are several Clq inhibition assays in which immune complexes in test sera inhibit ^{125}I -Clq from binding to some substance, i.e. sepharose (117), latex particles (118), or red blood cells (119). In general, these assays are more prone to interference than the direct binding assays.

Complement levels can also be used to evaluate the presence of immune complexes indirectly by determining the anticomplementarity of the sera using complement fixing (120). Interference in this assay

could be caused by alternate pathway activators (endotoxin, C3, nephritic factor, enzyme splitting complement component C1, and plasmin) and activators of C1 (C-reactive protein, heparin, and DNA). Low levels of complement can provide evidence for immune complexes but a low complement profile is not conclusive evidence for immune complexes. Further studies of complement breakdown products are necessary to demonstrate complement activation in the face of normal or elevated complement levels (121, 122, 79).

The Raji cell assay developed by Theofilopoulos et al (77) employs immune complex-bound complement reacting with the C receptors on the cell surface. Raji cells are from a lymphoblastoid line of cells which have Fc and complement receptors but no surface immunoglobulins (123, 124). Immune complexes which bind to the cells are detected using radiolabeled anti-human IgG with enough excess to flood the Fc receptors (77). Therefore, differences in radioactivity on Raji cells incubated with pathologic versus control sera will correspond to the presence of immune complexes (77). Like the Clq assays, the Raji cell assay detects complexes that bind complement (those with IgG, IgM and possibly IgA). Monomeric IgG also binds to Raji cells to potentially cause false positives, but the high ratio of complement receptors to Fc receptors and the standardization of IgG in patient samples probably minimizes this source of error (77). High levels of complement components C3d and C3 may displace complexes from the cells, and low complement levels may cause false negatives. Finally, the presence of antilymphocyte antibodies may result in false positives.

Assays Involving Fc Receptors

Immune complex assays which involve binding to the Fc receptor on

cells potentially have high sensitivity but no successful method for measuring direct Fc receptor binding has been reported. Consequently, immune complex assays involving binding to the Fc receptors all require the measurement of active cellular function stimulated or inhibited by immune complexes. Therefore, the assays must include standardization for cell metabolism as well as other parameters. Although complexes have a greater affinity for cell Fc receptors, uncomplexed immunoglobulins will bind on the receptors making standardization of immunoglobulin concentration in test sera necessary.

- In the platelet aggregation test (PAT) dilutions of test sera are used to aggregate platelets on microtiter plate (125). Interference is caused by platelet surface antibody, polyanions, and proteolytic enzymes, and some myxoviruses (126).
- In the K cell cytotoxicity assay blood lymphocytes are incubated with ^{51}Cr labeled target cells sensitized with rabbit antibody (127). Cell lysis and release of ^{51}Cr are inhibited by immune complexes but also to some extent by uncomplexed serum immunoglobulins and antilymphocyte antibodies (127).
- In the macrophage inhibition assay peritoneal exudate cells are incubated with test sera and radiolabeled aggregated IgG (128). Immune complexes in test sera will inhibit phagocytosis of the radiolabeled aggregates. The presence of anti-IgG might enhance phagocytosis and induce error in this assay (128).

Assays Involving Rheumatoid Factor or Bovine Conglutinin

Rheumatoid factor (RF) and bovine conglutinin are both substances with specificity for immune complexes that do not involve binding via

the usual complement or Fc receptor. Rheumatoid factor can either be of the IgG type or IgM type anti-IgG. Bovine conglutinin is a high molecular weight protein that binds immune complexes with fixed complement, especially C3d (129).

The RF assays are all inhibition assays using mRF or pRF except for the direct precipitation of RF and immune complexes in agarose gel (55). The selection of RF is very important because RF binding depends on the patient it was obtained from, and it is necessary to study a number of patients to find a RF with the greatest specificity and sensitivity. Monoclonal RF (mRF) obtained from patients with lymphoproliferative disease is generally more efficient than polyclonal RF (pRF) (55). Using the RF assays, positive results were observed in a majority of sera from patients with rheumatoid arthritis (57) but not patients with other diseases, i.e. systemic lupus erythematosus (55).

- In the assay developed by Luthra et al (57), immune complexes in test sera inhibit mRF conjugated to microcrystalline cellulose from binding to radiolabeled aggregated immunoglobulins.
- In the mRF assay developed by Lurhuma et al (129), immune complexes inhibit mRF aggregation of latex particles coated with IgG. One limitation is the fact that the presence of anti-IgG in test sera may mask the detection of the complexes. In addition to heat inactivating test sera prior to the assay, the assay must also be standardized for the concentration of IgG in the test sera because monomeric IgG can compete with complexes for the binding sites on RF (129).
- In the mRF assay by Gabriel et al (68), immune complexes inhibit ^{125}I mRF from binding to sepharose. This assay avoids interference by poly-

clonal factors in patient sera.

- In the pRF assay by Crowder et al (130), immune complexes inhibit pRF from binding to radiolabeled aggregated IgG which is then precipitated by antihuman IgM. The presence of anti-IgG in test sera may cause interference in this assay.

The bovine conglutinin assay involves incubation of radiolabeled aggregated IgG and test sera in polypropylene tubes coated with conglutinin (131). Immune complexes in the test sera will inhibit conglutinin binding to the labeled aggregates. This assay has many false negatives which may be due to insufficient complement in some test sera.

The ideal antigen-nonspecific assay would be specific, sensitive, and simple. The specificity is often difficult to access since all assays will give false positives with aggregated IgG. To ensure against this error, heating sera over 48°C and unnecessary freezing and thawing should be avoided. Evidence of assay specificity for immune complexes can be obtained by standardizing the assay against aggregated IgG, by comparing the assay results from patients with known disease with the results from controls, and by analyzing the size of the material identified in the assay -- immune complexes are larger than monomeric IgG.

The sensitivities of various assays are difficult to compare because few descriptions of assays report the size of IgG aggregates used in the standardization. The Raji cell assay, for instance, has greater sensitivity for large complexes formed near the equivalence point (77). On the other hand, some assays have greater sensitivity for a particular type of complex, i.e. the mRF assay for complexes found in rheumatoid arthritis (57). At the present time immune complex assays cannot be considered quantitative and their sensitivities must be based on their ability to correlate with clinical disease.

II. METHODS AND MATERIALS

Patient Selection, Sera Collection, Storage of Sera

The Clq reactivity of 46 sera and 10 joint fluids from 30 patients -- between the ages of three and 67 years -- was demonstrated using the ^{125}I -Clq binding assay. The diagnosis of Lyme arthritis was based on patients having the unique skin lesion of ECM, typical attacks of oligo-arthritis, and residency in the endemic area. Patients were evaluated for signs and symptoms at each clinic visit and placed in one of the following categories [1] Skin: patients with only ECM. [2] Joints: patients with arthritis, ECM still present or not, but no other systems involved. [3] Heart: patients with cardiac conduction abnormalities, regardless of ECM, arthritis, or peripheral neuropathy. [4] Central Nervous System: patients with meningitis or meningoencephalitis (documented by abnormal cerebrospinal fluid), regardless of all other features. [5] Peripheral Nervous System: patients with a peripheral neuropathy, without central nervous system or heart involvement, and regardless of skin or joint involvement. [6] Remission: patients with no symptoms or signs for at least two weeks. In order to define the relationship of immune complexes to the clinical course of Lyme arthritis, an additional 23 and 11 sera samples were obtained from two chronically ill patients, an 8-year-old boy and a 36-year-old woman respectively.

At each clinic visit, blood to be tested for immune complexes was drawn in Vacutainer tubes (Becton, Dickinson and Co., Rutherford, New Jersey) and allowed to clot for 1 hr. at 25°C. After centrifugation the serum was collected, stored in aliquots at -70°C in polypropylene vials (Electro Sonic Co., Hawthorne, California) and thawed a single

time for assay. The serum was stored a maximum of ten months prior to assay.

Isolation of Clq

Clq was isolated by the method of Yonemasu and Stroud (132).

Day 1: Blood from fasting donors was allowed to clot at 25°C for 1 hour and then stored for 1 hour at 4°C for clot retraction. Serum was separated by centrifugation at 1000 X g for 30 minutes at 4°C. About 100 ml of serum was combined with 25 ml of 0.1M disodium ethylene diamine tetraacetic acid (EDTA; pH 7.5) and incubated for 10 minutes at 37°C. The solution was cooled to room temperature and adjusted to pH 7.5 using NaOH and to a relative salt concentration (RSC) of 0.040 by titrating with 0.005M EDTA (pH 7.5). The solution was incubated with stirring for 1 hour at 4°C. After centrifugation at 10,000 X g (Servall centrifuge with GSA rotor) for 30 minutes and removal of the supernatant, the precipitate was washed twice with 0.1M EDTA buffer (pH 7.5, RSC = 0.04) using twice the original volume of the serum. The precipitate was dissolved in 12 ml of 0.01M EDTA buffer (0.75M NaCl, pH 5.0).

Day 2: The mixture was centrifuged at 25,000 X g for 30 minutes. The supernatant was collected, placed in dialysis tubing with a permeability of 24 Angstrom (VWR Scientific Co.) and dialysed overnight against 2 litres of 0.1M EDTA (pH 5.0, RSC = 0.078) at 4°C.

Day 3: The dialysing buffer was changed for 2 litres of fresh buffer, and dialysis was continued for 3 hours. Contents of tubing were collected and centrifuged at 10,000 X g for 30 minutes at 4°C. The supernatant was discarded and the precipitate was washed twice with 0.01M EDTA (pH 5.0, RSC = 0.078). The precipitate was redissolved in 0.005M EDTA

(pH 7.5, 0.3M NaCl) and refrigerated overnight.

Day 4: After the mixture was centrifuged at 20,000 X g for 30 minutes in the cold, the supernatant containing purified Clq was collected and stored at -70°C until used. The isolated Clq readily agglutinated latex particles coated with aggregated IgG but did not react with anti-sera to IgG, IgA or IgM in double diffusion in 1% (wt/vol) agarose gels.

Labeling Clq

Two hundred µg of isolated Clq was radiolabeled with ^{125}I (New England Nuclear Corp., Cambridge, Mass.) by a modification of the lactoperoxidase technique (133). First the Clq solution was dialyzed in phosphate buffered saline (PBS; pH 7.4, 0.01M PO_4 , 0.15M NaCl) overnight. To 100 ml of mixture (protein concentration 1 mg/ml), 6 µl of lactoperoxidase (protein concentration 1 mg/ml) was added. A 0.1M solution of potassium iodide was diluted 1:5000 in PBS and then added to the reaction mixture.

Under a hood ^{125}I (100 mCi/ml) was diluted five-fold in PBS, and 10 µl of diluted ^{125}I (a total of 200 mCi) was added to the above mixture containing Clq, lactoperoxidase and KI. The reaction was started by adding 10 µl of H_2O_2 (1:000 dilution of 30% H_2O_2 in PBS). After incubating for 20 minutes the reaction mixture was stopped with 1 ml of PBS. The mixture was dialysed for 4 hours against veronal buffer solution (VBS, .02M Na barbital, 0.25mM CaCl_2 , .75mM MgCl_2 , pH 7.3). The VBS was replaced with fresh buffer and the dialysis continued overnight. The ^{125}I -Clq had a specific activity of 0.6 to 0.9 mCi/µg of protein; and 10 µg aliquots were stored in 100 ml of VBS at -70°C. Prior to each assay, an aliquot was thawed, diluted to 5 ml with VBS containing 1%

bovine serum albumin (A grade Cal biochem, San Diego, California) and centrifuged at 20,000 X g for 40 minutes to remove aggregated protein.

The ^{125}I -Clq Binding Assay

The assay was performed in triplicate as described by Zubler et al (56).

Serum: For each test, 50 μl of unheated serum was combined with 100 μl of 0.2M EDTA (pH 7.5 adjusted with NaOH) in Bio Vial polypropylene tubes (Beckman Instruments, Inc., Palo Alto, California). The mixture was transferred to an ice bath, and 50 μl of ^{125}I -Clq solution was added immediately by 1 ml of 3% (wt./vol.) polyethylene glycol (PEG; Fisher, Pittsburgh, Penn.) in borate buffer (0.1M boric acid, 0.25 M di-sodium tetraborate, 0.075M NaCl, pH 8.3). After the addition of PEG, the mixture was incubated for 60 minutes at 4°C and then centrifuged in the cold for 20 minutes at 1,300 g. Under these conditions, Clq binds to the immune complexes while unbound Clq remains in solution. Precipitated radioactivity was determined in an automated gamma spectrometer (Biogamma 400, Beckman Instruments, Palo Alto, California). The precision and reproducibility of this method were tested using positive control serum from a patient with rheumatoid vasculitis and cryoglobulinemia. The coefficient of variation was 4% among 10 aliquots tested on the same day, and 11% among 7 aliquots tested on separate days.

Synovial Fluid: Synovial fluids, collected in tubes containing EDTA (final concentration 20 mM) were tested in the same manner but were diluted 1:2 in NHS prior to testing. When tested in this manner, Clq binding in synovial fluid from patients with osteoarthritis was not significantly different from NHS.

Sucrose Density Gradient Ultracentrifugation

The relative molecular size of the Clq reactive material was determined by fractionating sera on 10-40% linear sucrose density gradients in PBS (pH 7.4) using a Spinco model 65-75 ultracentrifuge with a SW 50.1 Ti rotor. Test samples were diluted 1:3 with PBS and 200 μ l aliquots were applied to 4.5 ml gradients and sedimented at 192,000 g for 14 hours. Individual 0.25 ml fractions were then collected from the bottom of each tube and analyzed for immune complexes. The location of IgG (7S marker) and IgM(19S marker) was determined by double diffusion in 1% (wt./vol.) agar gel.

Analysis of Results

Clq reactivity of sera and joint fluid from patients with Lyme arthritis was compared directly with sera from normal donors. Three of the 50 (6%) sera from normal donors surveyed with the ^{125}I -Clq binding assay had Clq reactivity 2 to 6 standard deviations from the mean. These sera were presumed to have excess reactive material -- immune complexes -- and therefore excluded. The remaining sera had reactivity approximating a Gaussian distribution (figure #1). The range of Clq binding in a panel of 10 normal sera was determined at each assay. Pathological sera with Clq binding greater than two S.D. from the mean of normal human sera (NHS) were considered to contain immune complexes.

Measurement of Complement

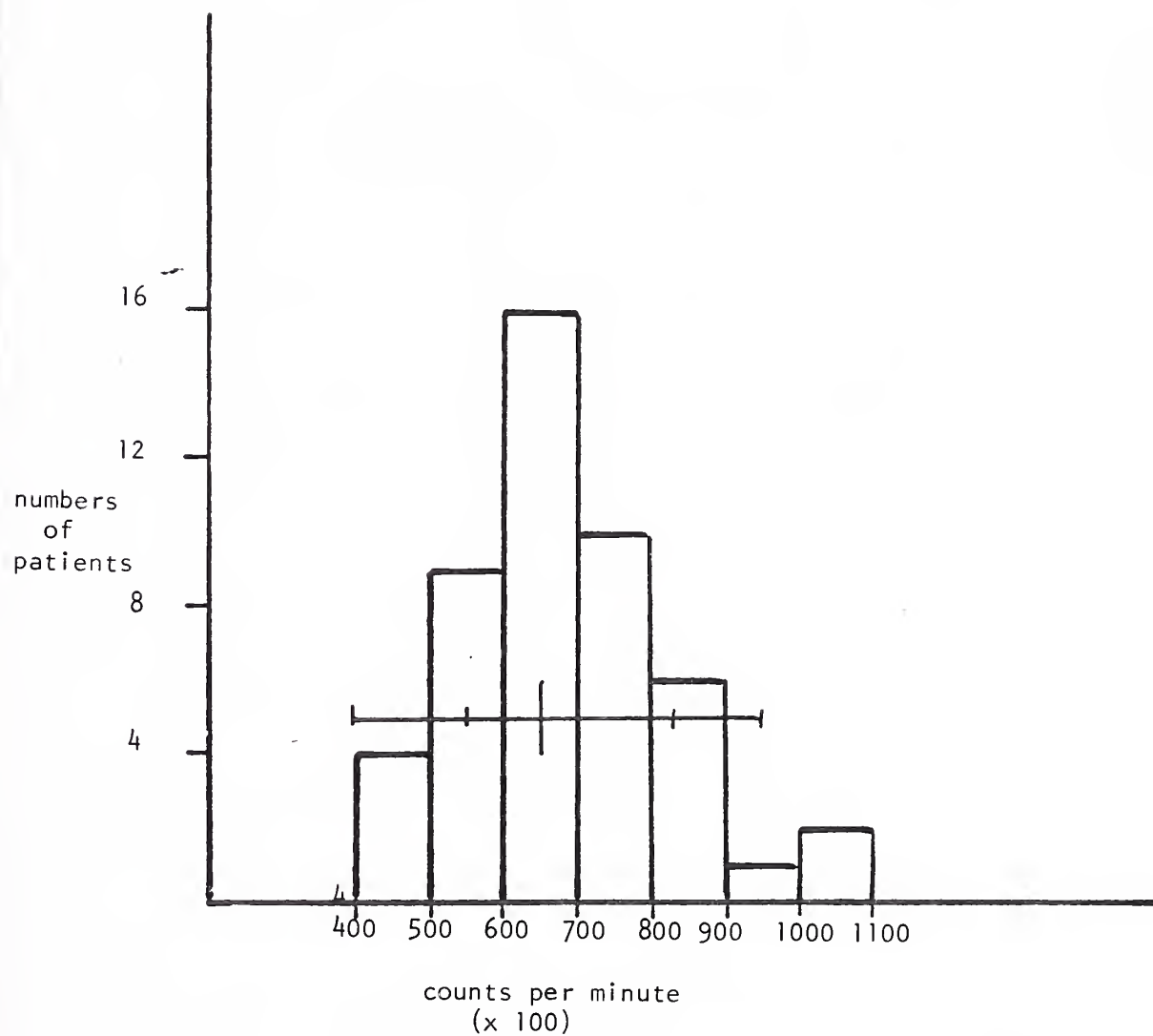
The complement determinations were performed in the laboratory of Dr. S. Ruddy. Total hemocytic complement (CH50) was measured by the method of Kent and Fife (134) and the third (C3) and fourth (C4) components by radial immunodiffusion in Hyland Plates (Hyland, Div. Travenol

Labs., Inc., Costa Mesa, California).

Measurement of Cryoglobulins

Blood for cryoglobulin determination was clotted at 37°C for one hour. The serum was collected by centrifugation at 400 X g for 20 minutes, and then 5 ml was allowed to stand for 72 hours at 4°C. Any precipitate that formed was sedimented at 1000 X g for 20 minutes in the cold, washed three times with cold PBS (pH 7.4, 0.01M PO₄, 0.15M NaCl), and resuspended in 0.5 ml PBS at 37°C. The total protein was measured by the method of Lowery et al (135).

FIGURE #1

 ^{125}I -Clq BINDING OF NORMAL SERA

III. RESULTS

Standardization of the ^{125}I -Clq Binding Assay

Aggregates of IgG were formed by dissolving lysophilized IgG in phosphate buffered saline (PBS; pH 7.4) and heating the solution at 63°C for twenty minutes. The solutions with aggregated IgG were then diluted 1:3 in normal human serum. The standard curve (figure #2) shows a linear relationship between the concentration of the aggregated IgG and the reactivity of the sample. This relationship remains linear down to a concentration of 120 $\mu\text{g/ml}$ of the aggregates. In the 50 μl aliquot of serum that is used for the assay, a minimum of about 2 μg is reactive in the assay. This sensitivity agrees with other reports of the Clq binding assay sensitivity (56) and with reports of the sensitivities of other assays, i.e. the Raji cell assay (77), and the rheumatoid factor inhibition assay (117).

Measurement of Immune Complexes

I measured immune complexes in 44 sera from 30 patients with Lyme arthritis using the ^{125}I -Clq binding assay (table #1). Among sera from patients with active disease 26/37 (70%) gave positive reactions. In contrast only 1/7 (14%) sera from patients in remission was positive in the assay. Only 9/14 (64%) of patients with arthritis had positive results in the assay (table #2). However, patients with extra-articular symptoms were frequently positive. Patients with ECM nearly always (10/11, 91%) had positive results in the assay, and both patients with nervous system involvement had positive results in the assay. The sickest patients, those with nervous system involvement, had sera with the greatest

Clq reactivity. Two or more sequential samples were obtained from 7 patients over a period of up to 8 months. These samples tended to remain either consistently positive or negative in the ^{125}I -Clq binding assay.

All 11 of the synovial fluids from 10 patients with Lyme arthritis were positive in the ^{125}I -Clq binding assay (table #3). However, only 6/11 of the sera samples obtained from these patients were positive in the assay.

Measurement of Complement

Complement levels were tested by measuring CH50, C3, and C4 in 15, 30, and 26 of 44 sera respectively (figure #3). Levels of CH50 were normal or elevated, and the levels of C3 and C4 were each below normal on only three occasions. Many patients, particularly those with arthritis, had complement levels above normal. In general, complement levels did not correlate with the ^{125}I -Clq reactivity of sera.

Physiochemical Properties of Immune Complexes

Analysis of complex size on sucrose density gradients demonstrated a correlation between complex size and disease manifestations. Sera from patients with early mild disease (ECM alone) and those from patients with more severe disease were fractioned and tested by the ^{125}I -Clq binding assay (figure #4). Complexes of 7S and 19S size were present in both patient groups but patients with severe disease had a predominance of the 19S complex while those with mild disease did not. The presence of ^{125}I -Clq reactivity with a substance of 7S size was difficult to account for because that size corresponded to a single IgG or a small antigen-a size which would be too small to fix complement (136). However,

FIGURE #2

^{125}I -Clq BINDING OF AGGREGATED IgG

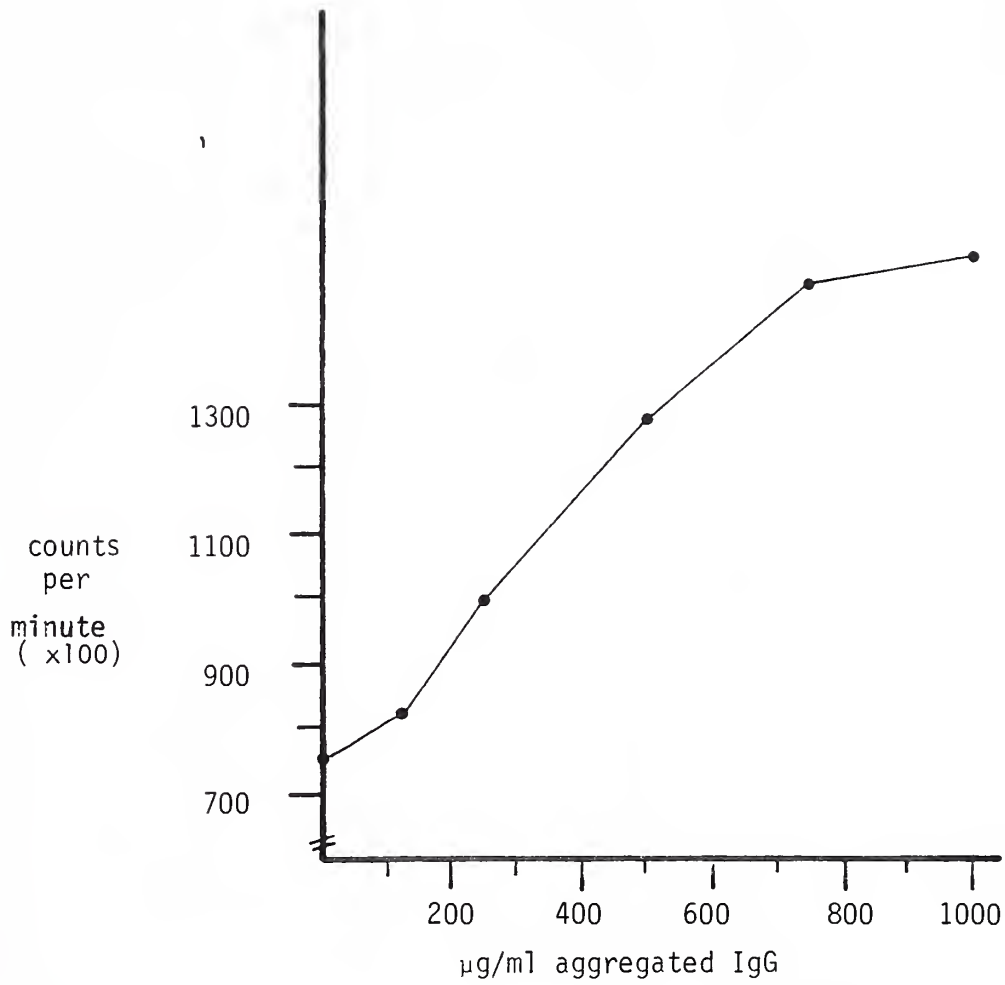


TABLE #1

COMPARISON OF Clq BINDING AND COMPLEMENT LEVELS

IN PATIENTS WITH LYME ARTHRITIS

Clinical Activity	Pt.	Intervals Between Sequential Samples (Mos.)	125 I-Clq Binding*	CH50 (units)	C3 (µg/ml)	C4 (µg/ml)
Skin	A		2.3	ND	2232	424
	B		4.2	ND	89	ND
	C		3.3	ND	ND	ND
	D		6.9	ND	ND	ND
	E		8.7	ND	ND	ND
	F		3.7	ND	ND	ND
	G		3.3	ND	ND	ND
	H		4.9	ND	89	ND
	I		2.8	ND	ND	ND
	J		-	ND	ND	ND
	K		2.0	ND	ND	ND
Joints	L		4.1	ND	2814	431
	M		2.8	214	1252	163
	"	1	5.5	258	1634	214
	N		2.3	ND	2306	267
	O		2.4	ND	2383	464
	"		-	ND	ND	ND
	P		-	207	1125	177
	"	1	4.6	ND	ND	ND
	Q		2.5	407	2232	630
	R		-	ND	1997	445
	S		2.0	304	2232	543
	T		-	341	3031	543
	U		2.1	335	2323	261
	V		-	338	1270	ND
	"	8.5	-	ND	1834	303
	"	0.25	-	ND	ND	ND
	W		-	ND	ND	ND
	X		2.0	314	3231	550
	Y		2.0	ND	80	ND
	"		3.5	ND	ND	ND
Central Nervous System	Z		15.4	171	1543	300
	"	2	15.8	153	1900	261
	"	2	6.9	236	761	290
	"	3	7.7	ND	798	240
	AA		6.0	ND	ND	ND
Heart	R		-	ND	1997	445
Remission	BB		-	175	2015	471
	CC		-	175	1551	308
	U		-	ND	2338	238
	"	4	-	259	1706	177
	DD		-	ND	1724	261
	EE		-	ND	1300	230
	FF		2.6	ND	ND	ND

*S.D. above mean of normal controls

(-) denotes results within the range of normal

[Complement values were determined in the laboratory of
Dr. S. Ruddy]

TABLE #2

Clq Reactive material During Different Phases of the Illness

	ECM*	<u>Latter Attacks</u>		
		Nervous System	Joints	Remission
Patients Tested	11	2	14	7
Number Positive (%)	10 (91%)	2 (100%)	(9) (64%)	1 (14%)

*Within three weeks of onset

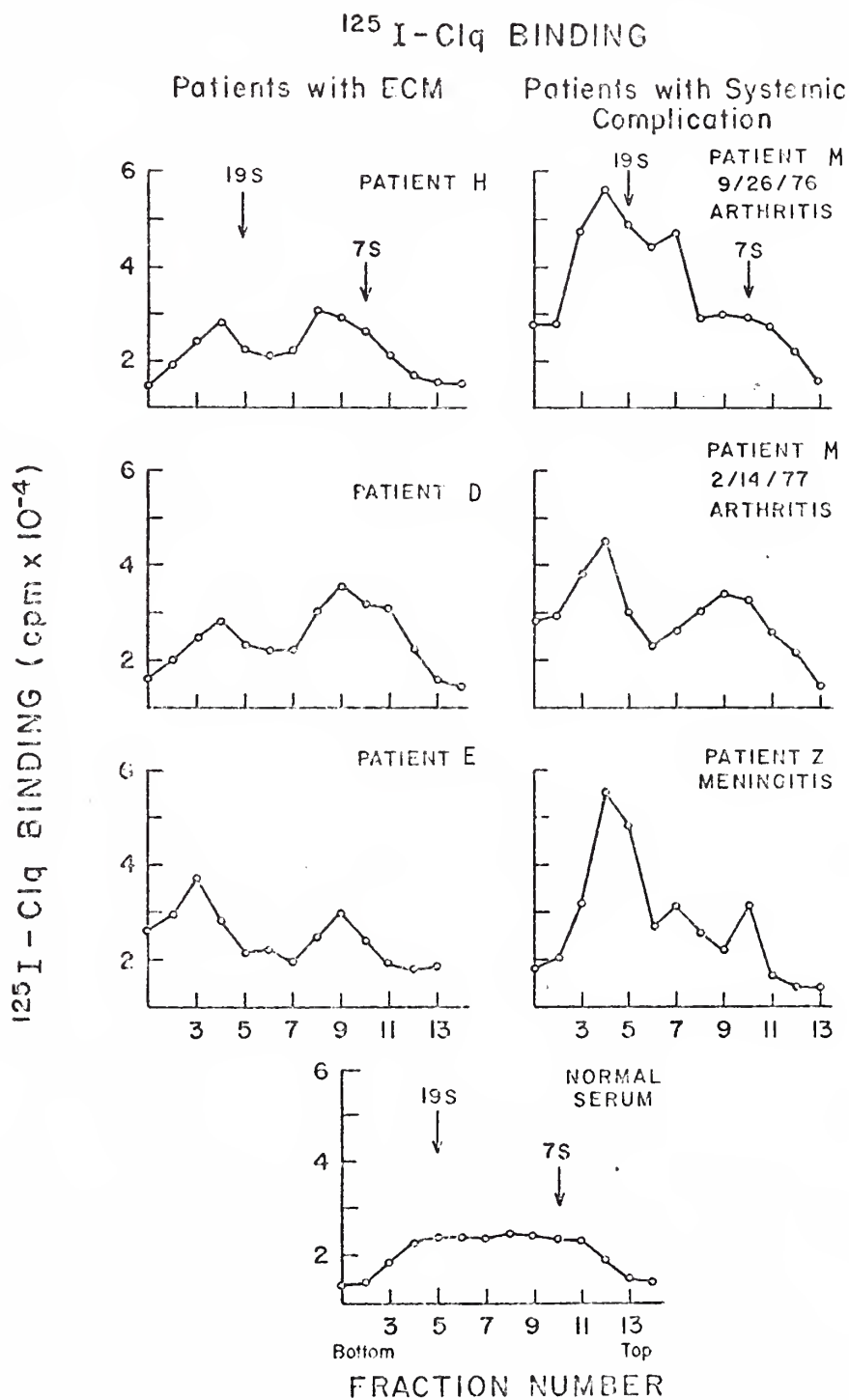
TABLE #3

Clq Binding* in Serum and Synovial Fluid

<u>Patient</u>	<u>Serum</u>	<u>Synovial Fluid</u>
A	NL	8.6
B	NL	9.6
C	NL	6.4
D	6.4	4.9
E	3.8	5.6
F	1.5	3.7
G	NL	7.9
H	3.1	2.2
"	4.5	6.6
I	5.0	20.0
J	8.1	6.0
Total	11	11
Number		
Positive(%)	7 (64%)	11 (100%)

 *Results are expressed as S.D. above the
 mean of normal sera.

FIGURE #4



the 7S material was not present in normal sera, and Clq reactive material of 7S density has been demonstrated in the sera of patients with systemic lupus erythematosus (56).

Some serum cryoglobulins from Lyme arthritis patients contained ^{125}I -Clq reactive material (table #4). Blood from six patients with Lyme arthritis and cryoglobulins was allowed to clot at 37°C for 30 minutes. The serum was removed and an aliquot was tested for ^{125}I -Clq reactivity. Cryoglobulins were precipitated by storing the sera at 4°C for 24 hours. The cryoprecipitable proteins were removed by centrifugation and then redissolved in a starting volume of normal serum. Both the supernatant (serum minus cryoglobulins) and the redissolved cryoprecipitate were tested. Five of the six sera had a decrease in Clq reactivity. The three sera with the most Clq reactivity transferred the reactivity to normal sera.

Patient Studies

I investigated the relationship of circulating immune complexes to the course of Lyme arthritis by following two patients for about nine months during various phases of their illness using the ^{125}I -Clq binding assay (figures #5, 6). The extent of ^{125}I -Clq binding activity tended to parallel the clinical course of these patients. One patient, an eight year old boy who was the sickest of the patients tested, illustrated the correlation between Clq reactivity and disease activity.

His initial clinic visit was July 1976 when he presented with an expanding skin lesion, headache, stiff neck and vomiting. Serum analysis revealed cryoglobulins composed of IgM with normal complement levels. ECM faded though mild symptoms persisted over the next month. On 26

TABLE #4

Clq Reactivity* of Cryoglobulins from Patients with Lyme Arthritis

Patient	Patient Serum		Normal Serum+
	Whole	Minus Cryo-globulins	Plus Redissolved Cryoglobulins
1	7.8	2.4	2.5
2	7.7	6.7	3.2
3	5.6	1.6	3.3
4	2.5	1.4	.4
5	2.1	2.7	.4
6	2.0	1.5	.6

125

* I-Clq binding assay, expressed as S.D. above the mean of normal sera.

+Clq reactivity 0.4 S.D.

FIGURE #5

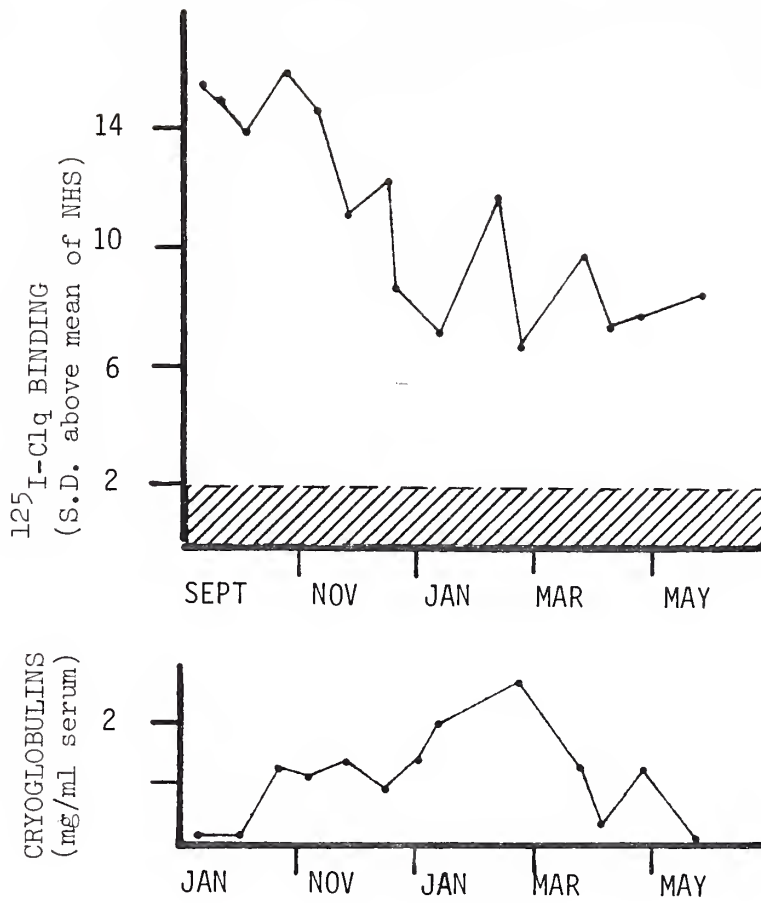
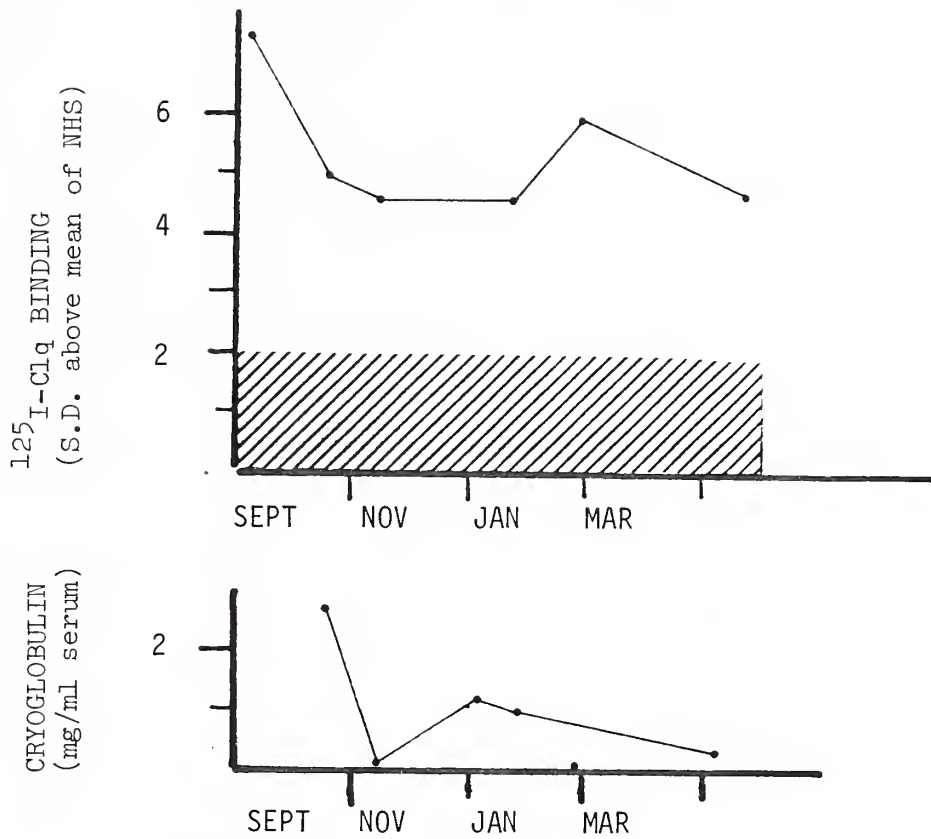
[illegible]

FIGURE #6



FEVER	+				+
NEURO					+
LYMPH		+		+	+
JOINT	+	+	+	+	+
JOINT		+	+	+	+
MYALGIA	+				
HEART					

August the patient had his worst fever in addition to headache, stiff neck and left shoulder pain. Serum cryoglobulins contained IgM and IgG with low normal complement levels. Oral prednisone was begun and the symptoms improved.

Beginning on 10 September he had serum samples analyzed in the ^{125}I -Clq binding assay (figure #5). In September and October he had moderate exacerbations of symptoms -- including fevers, joint pain, and adenopathy -- which correlated with peaks of Clq reactivity. Symptoms of only mild adenopathy and occasional headaches in November were accompanied by declining Clq reactivity of serum. In mid-December he developed choreiform movement of his hands with electroencephalogram changes followed by a sudden onset of pain and swelling in his right knee. These symptoms correlated with another peak of Clq reactivity of his serum. These acute symptoms resolved but the patient was left with a persistent arthritis -- diminishing over the next several months. A peak in Clq reactivity preceded the reappearance of the skin lesion in February and coincided with the onset of fever in March. The patient clinically improved and had no further peaks of Clq reactivity of serum, and by May he was nearly asymptomatic although his tests for ^{125}I -Clq binding activity were still positive.

IV. DISCUSSION

Lyme arthritis is a new disease of undetermined etiology. Although this disease was initially misdiagnosed as rheumatoid arthritis or juvenile rheumatoid arthritis, it has several clinical and laboratory features which are now recognized that set it apart from these disorders. Twenty-five percent of Lyme arthritis patients do satisfy the American Rheumatism Association's definition of juvenile rheumatoid arthritis (1) which is 6 weeks of monoarticular arthritis or polyarticular arthritis if other symptoms (i.e. fever, rash, or morning stiffness) are present or 3 months of arthritis if other symptoms are absent (137). However, the seasonal onset and the geographical clustering of Lyme arthritis distinguish this disease from juvenile rheumatoid arthritis. Unlike rheumatoid arthritis, Lyme arthritis is usually asymmetric and affects mostly large joints. In addition, antinuclear antibodies, rheumatoid factors or rheumatoid nodules have not been observed in Lyme arthritis. Two patients with Lyme arthritis developed carditis and fulfilled the Jones's criteria for acute rheumatic fever. However, in those two patients, the diagnosis of rheumatic fever could be excluded because of the lack of prior streptococcal infection detectable even by rising plus anti-streptococcal antibody titers, the presence of ECM as opposed to erythema marginatum, and the lack of significant response to aspirin (2).

Several clinical and laboratory features suggest that immune complexes are present in Lyme arthritis. The short attacks of arthritis, peripheral neuropathies, cardiac conduction defects and involvement of the central nervous system resembles known immune complex diseases such as serum sickness. Moreover, some patients have cryoglobulins in their

sera. As demonstrated by Steere et al (1) patients with ECM and cryoglobulins are more likely to go on to develop arthritis.

I have demonstrated that the sera and synovial fluids from patients with Lyme arthritis often react positively in the ^{125}I -Clq binding assay. The reactive material sedimented at 19S -- a density consistent with immune complexes involving polyvalent antigens. Further verification of the assay specificity for immune complexes was obtained by testing the reactivity of normal human sera with and without the addition of aggregated human IgG (agg. IgG). Only three of the 50 (figure #1) normal sera reacted in the assay whereas sera with IgG aggregates reacted in the assay with a linear relationship between the concentration of agg. IgG and the reactivity of the sample. A minimum of about 2 μg in the assay aliquot was reactive. Zubler et al (56) reported a similar sensitivity of the ^{125}I -Clq binding assay using agg. IgG and complexes composed of human IgG and rabbit anti-human antibody.

Furthermore, the Clq reactive material was disease related. Though reactive material was hardly ever present in normal sera, 27 of 44 sera from Lyme arthritis patients reacted positively in the assay. This reactive material was frequently present in the sera of patients with active disease 26/37 but rarely in patients with inactive disease 1/7. The sickest patients, those with central nervous system involvement, had the greatest amounts of reactive material in their sera. Patients with systemic involvement, i.e. ECM or nervous system involvement, more frequently had sera with positive tests than did sera from patients with symptoms localized to the joints. Similarly in rheumatoid arthritis, Zubler et al (56) reported that patients with systemic symptoms more

frequently had positive results in the ^{125}I -Clq binding assay than patients with arthritis alone. A longitudinal study of two patients with Lyme arthritis revealed that the Clq reactivity of sera rose and fell in parallel with exacerbations and remissions of disease. Changes in serum Clq reactivity in those two patients correlated best with changes in systemic rather than joint symptomatology.

Synovial fluids from Lyme arthritis patients also contained Clq reactive material while synovial fluids from patients with osteoarthritis did not. The synovial fluids reacted positively in the assay significantly more frequently 11/11 than did sera from the same patients 7/11. This observation was similar to the report by Zubler et al (56) that synovial fluids from rheumatoid arthritis patients were more frequently positive in the ^{125}I -Clq binding assay than were the sera samples. An explanation of this discrepancy between the Clq reactivity of serum and synovial fluid has been provided by Nydegger et al (63) with the demonstration that complement breakdown product, C3d, levels in serum and synovial fluids did not correlate. These results were consistent with parallel activation of complement in serum and synovial fluid and therefore parallel production of immune complexes. Consequently, patients with arthritis alone -- either because antigen production, antibody production, or both were localized to synovial tissues -- would not be expected to have marked elevations of serum immune complexes.

In short, I found that sera from Lyme arthritis patients with systemic disease frequently reacted and synovial fluids from patients with arthritis always reacted in the ^{125}I -Clq binding assay. These correlations between clinical features of Lyme arthritis and reactive material in

the sera and synovial fluids indicate that reliable measurements of disease-related substance, most likely immune complexes, were being made.

In a cooperative study with our laboratory; Hardin, Walker et al (138) confirmed the presence of reactive material in the sera of patients with Lyme arthritis using two other assays for immune complexes, the Raji cell assay and the Clq solid phase assay. The ^{125}I -Clq binding assay and the Clq solid phase results were usually concordant, and regression analysis demonstrated a strong positive correlation between the numerical values of the two assays. Furthermore, reactive material in the Clq solid phase assay also sedimented at 19S. In contrast, no correlation existed between the results of the Raji cell assay and either of the other two assays. Fewer sera had positive results in the Raji cell assay even though four sera were reactive in this assay alone. Similar discrepancies between the Clq solid phase and the Raji cell assay have been demonstrated in patients with gonococcemia (115) and glomerulonephritis (139).

The three assays all depend on the ability of immune complexes to fix complement, but differences in assay design may cause certain immune complexes to be preferentially detected in, and certain interfering factors to primarily affect, one assay. The ^{125}I -Clq binding assay will theoretically detect immune complexes consisting of any immunoglobulins which will bind complement and immune complexes of both large and small size. But this assay has less specificity with possible interference from C-reactive protein, DNA and endotoxin (111, 112, 113). The Clq solid phase assay is theoretically the most specific of the assays

because only complexes which bind Clq and staph protein A -- those with IgG1 or IgG2 -- will react in this assay (116). However, immune complexes with IgM, IgG3, or IgA may not be detected.

Part of the discrepancy between the results from the two Clq assays and the Raji cell assay may be explained by the differences in principle between them. The Raji cell assay detects complexes with bound C3 via cell surface complement receptors (140), whereas the Clq assays detect immune complexes that bind exogenous Clq (75, 139). Larger immune complexes formed near the equivalence point are preferentially detected in the Raji cell assay, and the assay is subject to a variety of interferences (77). Monomeric IgG and warm-reactive antilymphocyte antibodies might bind to these cells to cause false positives. In addition, the variations in complement levels may lead to erroneous results. Elevated levels of serum C3 may displace the cell-binding component of complement, C3b, from the cells. On the other hand, immune complexes in complement depleted sera may fail to react to the Raji cell assay.

The two Clq assays detected reactive material in about 45% of the sera from patients with active disease, and there was a remarkable correlation between the two assays ($p < .001$). These observations provided further evidence that immune complexes were present in patient sera, and possibly the results actually underestimated the true incidence of circulating immune complexes. The sera in the study had clotted at 25°C, a temperature at which some cryoprecipitable immune complexes become insoluble (141). Though the period of sera collection was short (about half an hour) and the temperature was well above 4°C, some Clq material may have been lost.

Many additional laboratory features suggest that immune complexes are involved in the pathogenesis of Lyme arthritis. For example, certain patients with Lyme arthritis have serum cryoglobulins which contain Clq reactive material (table #4). Patients with cryoglobulins have been noted to have depressed levels of complement consistent with consumption by immune aggregates (2).

The Clq reactive materials had compositions and densities consistent with those of immune complexes. The complexes were composed of IgG1 or IgG2 and complement to react in the Clq solid phase and Raji cell assays. Serum from Lyme arthritis patients had Clq reactive material in the lower and top portions of sucrose density gradients indicating the presence of reactive substances with 19S and 7S densities respectively. Similar 19S reactive substances have been reported in rheumatoid arthritis (56) and cytomegalovirus infections (142). Immune complexes of this size are likely to cause tissue injury (143), and it may be important that the largest amounts of this reactive material were detected in patients with severe clinical manifestations. The high density material was detected best in the Clq assays; thus it is not surprising that sera from patients with active disease were more frequently positive in the Clq solid phase and the ^{125}I -Clq binding assays than in the Raji cell assay.

The low density material was consistently reactive in the ^{125}I -Clq binding and Raji cell assays when patient sera was tested but not when normal sera was tested. Furthermore, sera from patients with systemic lupus erythematosus had Clq reactive material of 7S density in the ^{125}I -Clq binding assay (56). The relatively greater reactivity of this

material in the Raji cell assay might be explained by the presence of immune complexes with complement receptors already saturated or by the presence of antilymphocyte antibodies. The reactivity of the 7S material in the ^{125}I -Clq binding assay might be the result of antibodies directed against Clq. Low density complexes composed of small antigens and IgG or IgG alone might undergo sufficient conformational change with subsequent increase in density in order to precipitate after Clq binding and the addition of PEG. Antilymphocyte antibodies circulating as complexes with cell surface antigens might explain the reactivity of low density material in both the Raji cell and the ^{125}I -Clq binding assay. Winfield et al (144) have reported the presence of antilymphocyte antibodies in the serum cryoprecipitate of patients with systemic lupus erythematosus. Of the four patient sera that were fractionated and tested by the Clq solid phase assay, only two had reactive material of 7S density. The decreased reactivity of this material in the Clq solid phase assay might have resulted from the presence of complexes which only weakly bind staph protein A, i.e. complexes composed predominantly of IgG3.

The sera with reactive material did not appear to have depressed complement levels (figure #3) which usually follow the production of complexes unless there is increased synthesis of complement. In rheumatoid arthritis, for example, the complement levels are usually normal or elevated (59, 60, 61), yet Nydegger has demonstrated that the activated component of complement, C3d, was elevated in parallel with the ^{125}I -Clq binding activity (63). Patients with ECM and cryoglobulins have been noted to have depressed levels of complement (2). This

discrepancy between the normal complement levels in the presence of Clq reactive material and the depressed complement levels in the presence of cryoglobulins might be explained by a qualitative difference between the two substances. This difference was demonstrated by the fact that not all cryoglobulins transferred Clq reactivity (table #4).

V. SUMMARY

Immune complexes are present in a number of disorders where they are thought to be of pathogenetic importance. To this group can now be added Lyme arthritis, a newly recognized, cyclical, inflammatory disease thought to be transmitted by an arthropod vector. The disease affects predominantly skin and joints and to a lesser degree the nervous system and heart. Tests for antinuclear and rheumatoid factors have been negative, but some patients have serum cryoglobulins. Consequently, I looked for circulating immune complexes using the ^{125}I -Clq binding assay.

I tested 44 sera from 36 patients who had either active disease or a recent remission. Almost all the positive reactions occurred in the sera from patients with active disease 26/37 (70%) versus 1/7 (14%) with inactive disease. In addition the synovial fluids from 10 patients were all positive.

The densities of the complexes were determined by fractionating six sera from five patients on sucrose density gradients and analyzing them in the ^{125}I -Clq binding assay. This method demonstrated Clq reactive material of 19S and 7S densities. The larger 19S material predominated in patients with severe disease, and similar size complexes have been reported in other immune complex diseases (142). The identity of the 7S material and its relation to disease activity has not yet been determined.

This thesis demonstrates that patients with Lyme arthritis have circulating immune complexes. Their relationship to active disease and their physiochemical properties support a role for immune complexes in

the pathogenesis of the disease. Finally, the similarities of joint pathology and the presence of immune complexes in both diseases make Lyme arthritis an ideal model for studying rheumatoid arthritis.

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